

I claim:

1. A variant single chain human tissue-type plasminogen activator protein having R275
5 and at least one other basic amino acid residue in the serine protease region substituted by a non-basic amino acid residue thereby disrupting the salt bridge interaction between aspartate 477 and lysine 429.
2. The protein of claim 1 wherein the non-basic amino acid residue is chosen from the group consisting of glycine, serine, threonine, asparagine, tyrosine, glutamine, aspartic
10 acid, and glutamic acid and having a zymogenicity of at least 10.
3. The protein of claim 1 having a zymogenicity of at least 50.
4. The protein of claim 1 having a zymogenicity of at least 100.
5. The protein of claim 1 having a fibrin stimulation factor of at least 10,000.
6. The protein of claim 1 having a fibrin stimulation factor of at least 20,000.
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7. The protein of claim 1 having a fibrin stimulation factor of at least 10,000.
8. The protein of claim 2 having a fibrin stimulation factor of at least 20,000.
9. The protein of claim 3 having a fibrin stimulation factor of at least 20,000.
10. The protein of claim 1 wherein the protein is at least a factor of 5 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
20 protein.
11. The protein of claim 1 wherein the protein is at least a factor of 9 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
protein.
12. The protein of claim 1 wherein the protein is at least a factor of 200 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
25 protein.
13. The protein of claim 8 wherein the protein is at least a factor of 9 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
protein.
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14. The protein of claim 8 wherein the protein is at least a factor of 200 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
protein.
15. The protein of claim 1 wherein the protein has a fibrin selectivity factor of at least 100.
16. The protein of claim 8 wherein the protein has a fibrin selectivity factor of at least 100.

17. The protein of claim 14 wherein the protein has a fibrin selectivity factor of at least 100.
- 5 18. A polynucleotide encoding the protein of claim 1.
19. An expression vector comprising the polynucleotide of claim 18.
20. A cell comprising the expression vector of claim 19.
21. A variant single chain human tissue-type plasminogen activator protein selected from the group consisting of R275E,H417D, R275E,H417E and R275E,K429Y.
- 10 22. A polynucleotide encoding the protein of claim 21.
23. An expression vector comprising the polynucleotide of claim 22.
24. A cell comprising the expression vector of claim 23.
25. A composition for the treatment of an thrombotic condition comprising a physiologically effective amount of the protein of claim 1 in a pharmaceutically suitable excipient.
- 15 26. The composition of claim 25 wherein the dose of the protein is from about 0.05 milligram per kilogram body weight to about 0.2 milligrams per kilogram body weight.
27. A diagnostic kit comprising antibodies to the protein of claim 1.
- 20 28. A diagnostic kit comprising the protein of claim 1.
29. A diagnostic kit comprising polynucleotides capable of hybridizing to the polynucleotide of claim 18.
30. A method of making a variant single chain human tissue-type plasminogen activator protein comprising the steps of culturing the cell of claim 24.
- 25 31. The method of claim 30 further comprising the additional step of purifying the protein.